BACILLUS MUCOSUS CAPSULATUS.

A STUDY OF THE GROUP AND AN ATTEMPT AT CLASSIFICATION OF THE VARIETIES DESCRIBED.*

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SINCE Friedländer, in 1883, first called attention to the presence of capsulated bacilli in pneumonia, a number of observers have described organisms more or less similar in form, and found in a variety of pathological lesions. The tendency to describe bacteria as new, if they differed in any way from previously described organisms, has been carried to its widest limits in this group. Fricke, in his exhaustive monograph, has analyzed twenty-two varieties described under different names, and to these must be added two others, noted by observers in this country. All these organisms were conceded to have certain main characteristics in common; but inasmuch as they did not in all respects agree with their prototype, the pneumo-bacillus of Friedländer, they were described in detail, and given a number of names.

Of late the work of bacteriologists has tended rather to the grouping of closely allied bacteria as sub-varieties under a single head than to multiplying varieties on the ground of minute and often inconstant characteristics. The almost infinite capacity for variation in the appearance of sub-cultures from the same colony, if the circumstances of growth are changed even to a minor degree, is a clearly sufficient basis for such a tendency. The necessity for classification of species on permanent and universally found differences is something that need not be dwelt on, and the simpler such classification can be, coincident with clearness, the more valuable it will be, not only to the specialist along these lines, but to the more or less casual worker who is at present often lost among the multiplicity of detail.

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In three years' work as resident pathologist of the Lakeside Hospital, the writer was struck with the frequency of bacteria, apparently of the B. mucosus capsulatus group, in the routine autopsy plates. These were made at all autopsies from the heart's blood, the lungs, the liver, the spleen, and the kidneys, and from any other organs or fluids which showed changes indicating the possible presence of micro-organisms. In about one-fourth of all cases colonies of the B. mucosus capsulatus type appeared on the plates in sufficient numbers and in such varied distribution as to exclude accidental contamination. Plates were, however, made as a check, from the air of the different rooms where the bacteriological work was done, but in no case was an organism of this type recovered.

A piece of work was begun with the bacteria isolated, but, owing to certain accidents, these were lost, and the research remained incomplete. Enough has been recorded to establish the relationship of these earlier cultivated organisms with the later ones, and with the group under consideration.

According to Fricke, the general characteristics of the group are as follows: The organisms are short, possess capsules, show marked pleomorphism, and form no spores. They are not motile, and decolorize by Gram's method. They grow on a variety of media in a profuse slimy layer, and in gelatin slabs they do not liquefy, but show the so-called "nail-growth." In addition to this, most of the bacteria classed under this head form a moderate amount of indol, and ferment carbohydrates in solution with the formation of gas and acid.

In 286 consecutive autopsies in which routine cultures were made, organisms answering to the above description were isolated in 79 cases. In all but 5 of these the cultures came under my personal observation, and in the others the recorded descriptions are not sufficiently worked out to exclude the colon bacillus, and will therefore not be considered. This leaves 74 cases, or 25.8 per cent. of cases studied bacteriologically. The bodies of persons dying in the hospital are placed at once in cold storage at 0° C., and autopsies are almost always performed within twenty-four hours of death.

DISTRIBUTION.

The occurrence of B. mucosus capsulatus has been noted by many authors, and in a great variety of places and lesions. The literature in this respect has been so fully covered by Fricke in 1896, and Clairmont in 1902, that it seems scarcely worth while to do more than summarize the findings.

Outside of the animal body it has been found in the soil, in the air, in cracks in the floor, in dust, and in water. It has been found in human beings in health, in the nose frequently, in the mouth more rarely (according to Netter in 4.5 per cent. of cases), in saliva, and in the gastro-intestinal tract. In disease it has been isolated from the sputum of influenza patients, and of persons with pneumonia and tuberculosis, of course in association with other organisms. The pathological lesions in which it has been found may be divided according to their relations to the body functions:

In the *respiratory tract*, it has been found in rhinoscleroma, ozena, inflammatory conditions of the nasal passages and accessory nasal sinuses, in lobar and bronchopneumonia, in abscess and gangrene of the lungs, in bronchitis, and in bronchiectasis; in the *digestive tract*, in stomatitis, gastroenteritis, dysentery, and appendicitis; in *serous cavities*, in pleurisy of various types, in pericarditis, and in peritonitis, usually perforative in origin; in the *urinary tract*, in cystitis, acute and chronic, pyoureter, pyonephrosis, pyelonephritis, abscess of the kidney, and in infected adenocystoma of the kidney; in the *circulatory system*, in acute ulcerative endocarditis; in the *genital tract*, in acute endometritis; in the *nervous system*, in brain abscess, gas cysts of the brain, meningitis; in the *special sense organs*, in otitis media.

Besides these local infections, a number of cases have been described in which there has been a *general infection*, often of the hemorrhagic type, as noted by Howard, Blumer and others.

Distribution in Cleveland.—A number of attempts were made to ascertain the distribution in Cleveland. Plates from earth obtained along the streets in different parts of the city showed no organisms of this type. Investigations pursued independently by the class in bacteriology, and by the City Bacteriological Labora-

tory, failed to show it in city water. (Since the writing of this article, organisms of the group have been found in the city water supply, 1903.) Air plates in the laboratories of the Medical School, and those of Lakeside Hospital, were also negative in this regard. A series of tests by the City Laboratory in 1902 (repeated in 1903), showed none of this group in a large series of vaccine points and tubes from different makers.

In eighteen plates made from the throats of members of the class in bacteriology, all of whom considered themselves well at the time, only one showed cultures of the desired kind.

At eighteen successive autopsies at Lakeside, cultures were made from the stomach, small and large intestine, before these were opened, in the usual manner. In ten of these it was possible to isolate organisms of this group, the so-called "aërogenes," from small and large intestines, but in no case from the stomach. It is probable that the presence of the colon bacillus may in some of the other cases have obscured the colonies of B. mucosus capsulatus, so that the number of positive cases is perhaps larger.

The series of seventy-four cases from the Lakeside Hospital covers quite a wide pathological field, and the lesions in which the organisms were found may be summarized as follows:

Bronchopneumonia	19	Adenocystoma of kidney infected	1
Lobar pneumonia	1	Tubercular abscess of kidney	3
Hypostatic pneumonia	1	Peritonitis, purulent	6
Bronchitis	5	Pericarditis, purulent	1
Gangrene of lung	1	Endometritis, acute	1
Lung tuberculosis, cavity	3	Omphalitis, acute	1
Miliary tuberculosis of lung	1	Otitis media	1
Infarction of lung	1	Carcinoma of liver	1
Hemorrhage into lung	1	Gas cysts of brain, general gaseous emphysema	1
Cystitis	3		—
Pyonephrosis	2	Total	56
Pyelonephitis	2		
Pyoureter	1		

In 14 cases the organisms were found distributed through all organs, though none could be classed as hemorrhagic septicemias, the nearest approach being the one in which gas cysts of the brain and a general gaseous emphysema were observed. In the

other cases there were no lesions found to account for the presence of the organisms.

In hospital cases outside the autopsy service, bacteria of this type were found in three appendix cases in the surgical service, and in a small pustule on the face of one of the laboratory attendants. The organism belonging to this group which caused a gaseous and hemorrhagic epidemic among the laboratory guinea pigs has already been noted elsewhere, and comes under the first class in the final classification in this paper.

In the present piece of work, 37 organisms were studied, 19 of them being from the Lakeside material, and the other 18 from various sources, with the idea of comparing as many members of the group as possible under the same cultural conditions.

From Král's laboratory the following strains were secured: B. pneumoniæ Friedländer, B. capsulatus mucosus (Kruse, Pauling, Fasching), B. aërogenes Escherich, B. acidi lactici, B. crassūs sputigenus Kreibohm, B. pseudo-pneumonicus Passet, B. capsulatus Pfeiffer, B. ozænæ Abel, B. rhinoscleromæ; through the courtesy of Dr. Harris, of the Johns Hopkins Hospital, six strains isolated from various sources and classified as B. pneum. Friedländer; through the courtesy of Dr. Blumer, of Albany, B. mucosus capsulatus Blumer, described by him in a case of hemorrhagic septicemia; and from the stock cultures in my laboratory, B. hemorrhagic septicemia Howard, and B. mucosus capsulatus Wright and Mallory.

GENERAL TECHNIC.

The series was inoculated into plain, glycerin and glucose agar, gelatin, milk, dextrose-free and ordinary bouillon, potato, blood serum, and dextrose-free bouillon containing 1 per cent. of the various carbohydrates under consideration. Where possible, the media were made up according to the specifications of the American Public Health Association, and titrated to a reaction of + 1.5 to phenolphthalein.

The appearances on the different media will be taken up separately, with discussion of the results of other observers. In the tabulation it will be noticed that only twelve of the Cleveland cultures are analyzed. The other seven were so exactly like some of

those tabulated that it would be a useless repetition to set them down in full. B. m. c. 13, from kidney Aut. 238, was identical with B. m. c. 1; the kidney showed no lesion. B. m. c. 14, 15, 16, 17 were identical with B. m. c. 3; they were all from the lungs, which showed no pneumonia, but congestion and edema. B. m. c. 18, 19, were from the liver in one case and the spleen in another, and identical with B. m. c. 5; neither organ showed marked change.

GROWTH ON DIFFERENT MEDIA.

Plain agar.—The colony appearance on this medium was studied with a view of ascertaining whether the differences in the colonies of the different varieties were as constant and valuable as many writers state them to be. Strong has formed two main groups:

I. Young colonies colorless, older whitish, capsules present only in animal exudates and easily stained. In this class he places B. pneumoniæ Friedländer, Wright and Mallory, B. ozænæ Abel, B. crassus sputigenus, and perhaps rhinoscleroma.

II. Colonies white from the first, capsules hard to demonstrate. In this class come B. aërogenes, Pfeiffer, Kruse.

In plates from all the Cleveland series, except No. 6, the colonies were in general of the second type. In the cultures obtained elsewhere, B. aërogenes, B. capsulatus septicus, B. pseudo-pneumonicus, Fasching, Pfeiffer, Howard, and Blumer also came under this head.

Rhinoscleroma, and Wright and Mallory, were still pale at thirty-six hours, but Friedländer, B. ozænæ Abel, and B. sputigenus crassus, though pale at the very first, after twenty-four hours showed no essential difference from those of the second group.

It was very noticeable that colonies on the same plate often differed widely in appearance, both as to color and as to appearance under the low power. Further development of such colonies showed them to be similar, and plates made from any single colony showed the same variations. When the colony started just below the surface and broke through, or when the agar was a little dry, the tendency seemed to be toward the whiter color.

In general, the colonies were raised, white, moist, glistening, usually round, but at times with wavy borders, and were yellowish by transmitted light. Under the low power they were finely to coarsely granular, the coarser granules being usually at the center and shading off at the edges to an almost transparent border. No absolute essential and differentiating characteristics could be seen, agreeing with the observations of Fricke, Clairmont, and others. In my work the divisions made by Strong did not appear to be constant.

Glycerin agar slants.—The growths, while very characteristic of the group as a whole, showed no constant differences. In the majority of cases the growth was profuse, much wider at the bottom than at the top, moist and shiny, with a well-marked porcelain-like appearance. The edges were at times sharply defined, and at times shaded off into the media. The center line was sometimes raised, sometimes in the form of a shallow groove. The stab was granular, showed good growth all the way down, and in most cases there was profuse development of gas. The water of condensation was heavily clouded, and there was usually a large flocculent sediment. The viscidity varied, being absolutely constant only in B. rhinoscleromæ and in No. 9 of my series.

The growth of B. rhinoscleromæ was rather thin and paler than the rest, the water of condensation was always very viscid, and no gas was formed in the stab.

In general, the less moisture in the agar, the greater the tendency to a raised, sharply defined, rather narrow growth, which in media containing more water tends to spread out toward the sides and to be thinner, especially at the edges. The gas formation is much more extensive in fresh moist agar.

Glucose agar.—The growth on this medium showed nothing additional to the above, except that the organisms capable of fermenting gave more gas than in glycerin agar.

Löffler's blood serum.—The cultures on this medium were not characteristic. The growths were profuse, yellowish white, and could not be told apart when the labels were covered. No liquefaction ever took place and no especial odor was ever noted.

Gelatin.—Several series of tubes were inoculated at different times during the year, and observed over a period of four weeks each. They were kept at room temperature, and exposed to ordinary daylight, though not to direct sunlight. One series was made on media a week old; the others, on media within twenty-four hours from the last sterilization.

In the first series, where the media were a little more dry, the characteristic round, elevated, so-called "nail-culture" was more frequent than in the subsequent inoculations, suggesting that the amount of moisture in the gelatin may have a very definite influence over that phenomenon. Pfeiffer's bacillus, which, according to Clairmont, never forms the nail-growth, did form it in the dryer gelatin, though not in the other.

In general, in the 38 cultures examined, 15 had rounded tops in all inoculations, while 5 always grew in a flattened mass on the surface, with no suggestion of a nail-head. The other 18 varied in different series, never being very typical.

In the stab, 18 were very coarsely granular, without any branches projecting from the sides, while 8 were always finely granular, usually with fine feathery outgrowths. The rest were sometimes finely, sometimes coarsely, granular. The feathery outgrowths in the stem were constant in 16 and absent in 22. The irregularity of these appearances and their relations to the different organisms indicate that there can be no absolute dependence placed on the gelatin growth. Development of a brownish color in the media after long periods of growth—a phenomenon also observed in glycerin agar—was in both media variable and inconstant. The cloudiness just below the surface, observed by Herla, Wilde, and Scheffer in old cultures, but never seen by Clairmont, was quite frequent, but not constant in any case. When the gelatin was made from dextrose-free bouillon, no development of gas was ever seen.

Milk.—Inoculations were made into litmus milk several times, at wide intervals, and organisms that were passed through animals were tested again after recultivation, to ascertain if there were any changes.

Although there was a moderate variation in the time of coagu-

lation of different samples of milk, and although the amount of acidity varied somewhat, still those which coagulated in the first instance always coagulated, and those which did not coagulate in the first instance never did so. To this there was one exception, B. pseudo-pneumonicus Passet. This, when received from Král, neither coagulated milk nor fermented sugars, but after passage through animals was able to do both. The other organisms which, when received, did not ferment milk, were also passed through animals, but neither this procedure nor prolonged cultivation in milk made any difference in their powers.

The statement widely made, both in the original article and in a variety of text-books, that Friedländer's bacillus ferments lactose, but does not coagulate milk, is denied by Strong. Clairmont finds traces of gas formation from lactose, and no coagulation of milk, while Fricke finds both gas formation from lactose and coagulation of milk. As to actual gas formation, it was noted that, unless the sample of lactose from which the dextrose-free bouillon was prepared was chemically pure, traces of gas might be found. There is also a possibility that the bouillon was not completely dextrose-free. In all our trials of the organisms which were included in this type (Class II in the final classification) we never found any gas formation from lactose, nor any coagulation of milk. The reaction was, however, as noted below, slightly acid, and in the fermentation tubes there was a very slight cloud in the first twenty-four hours, which usually disappeared before the forty-eight-hour examination. It is therefore possible that there may be a very slight fermentation for lactose, though it seems more probable that in the case of the milk the acid reaction is due to the presence of some dextrose, and that traces were also present in the fermentation tube.

The average time of coagulation varied somewhat, most of the lactose fermenters causing a firm clot in from twenty-four to forty-eight hours. Pfeiffer's bacillus, as seems to be the observation of most writers, takes from forty-eight to seventy-two hours, and in the present series Nos. 5, 10–12, and Howard, showed the same characteristic.

Those which did not ferment lactose were observed with especial care and frequency, and at no time was there any coagu-

lation, though in many cases a small amount of acid formation was seen. Wright and Mallory, said in the original to coagulate slowly, gave acid, but no coagulation, as was also observed by Strong. No. 6, and B. sputigenus crassus also gave an acid reaction. Hopkins cultures Nos. 3, 4, and 6, and Friedländer, were amphoteric. Fasching and B. rhinoscleromæ were persistently alkaline.

Howard found that Pfeiffer, Howard, and a bacillus from the antrum coagulated in from forty-eight to seventy-two hours, Wright and Mallory in from twenty-four to forty-eight, and that a culture from Reed of Washington, made from the throat in a case of diphtheria, as well as the laboratory Friedländer, did not coagulate at all.

Examination of the recorded cases at Lakeside Hospital shows that of the seventy-four recorded cases, only four did not coagulate. One of these, re-examined in the present series, No. 1, does coagulate in from twenty-four to forty-eight hours, indicating that in the whole series not more than four can be said to belong to the type characteristic of Friedländer.

Clairmont divides his organisms into three groups according to the time of coagulation, but, in view of the variation found in different samples of milk, this distinction seems an artificial one, the only well-marked separation being between those which coagulate and those which do not.

Fricke classes as Friedländer several organisms which ferment lactose and coagulate milk, which does not agree with the original description by Friedländer.

Potato.—Much emphasis has been laid on the growth of this group of organisms on potato, and indeed Fricke and others have gone so far as to attempt a definite classification on this basis. Clairmont does not, however, share his views, and the appearance of the cultures in this series leads to the belief that, while it is a very favorable medium for the growth of the organisms, there are no constant characteristics which enable us to say whether we are dealing with any special member of the group.

Several series of potato were inoculated, and the differences of growth of the same organism on successive samples were very

striking. In one series there was insufficient water with the potato, and gas formation was very rare, while where more water was present this was a conspicuous feature.

On the moist potato, on which the growth is probably most typical, there were in general two types of growth.

I. Profuse, moist, yellowish, growing somewhat darker with age; surface shiny; edges sharply defined; gas bubbles in the growth in varying amount; odor aromatic. This type includes Nos. 1–5, 7, 8, 10–19, Hopkins Nos. 1, 2, B. pseudo-pneumonicus, Howard, Wright, and Mallory. Under this head may also be included No. 9, B. aërogenes, Pfeiffer, Blumer, and B. acidi lactici, which differed only in the variability of their gas formation.

II. Growth rather more thin and dry, often waxy in appearance; sometimes a few gas bubbles were seen, but usually there were none. This includes No. 6, Hopkins Nos. 3–6, Friedländer, Fasching, B. capsulatus septicus, B. ozænæ Abel.

B. rhinoscleromæ and B. sputigenus crassus gave a thin transparent watery growth spreading over the surface.

These appearances varied on different potatoes, but the average results are as above. To illustrate the variations possible on this medium, it is sufficient to note that, whereas Clairmont describes the growth of Wright and Mallory as thin and transparent, only to be seen by the shining appearance of the potato, it was found here to be the most active of all, with profuse gas formation. In his culture of B. rhinoscleromæ from Prague the same author obtained a somewhat raised, dirty growth, while my culture from the same source grew scarcely at all on this medium.

Discoloration of the potato also varied. In No. 2, Hopkins No. 3, B. pseudo-pneumonicus, and Blumer it was always marked after a few days, while it was never seen in B. capsulatus septicus, B. ozænæ Abel, or B. rhinoscleromæ. In all the others no rule could be made.

In spite of such discrepancies, Fricke contends that the growth on potato is sufficiently regular to admit of classification, and divides his organisms into two groups, as follows:

I. Yellow, profuse, sharply defined growth, of the consistency of thin salve; surface moist, irregular, glistening; growth soon

covering the surface, In this class he places Friedländer and Pfeiffer.

II. Profuse, moist, viscid growth, almost colorless, spreading rapidly; no discoloration; no gas formation. This includes B. ozænæ Abel.

In my series, Pfeiffer's bacillus and Friedländer always reacted differently from one another, but this difference from Fricke may lie in the fact, above stated, that his Friedländer acted on sugars in a manner similar to Pfeiffer, and was therefore probably not a Friedländer at all.

Bouillon.—Both dextrose-free bouillon, made up from different samples of meat, and ordinary bouillon, made up with Liebig's extract, were used with the organisms studied. The inoculated tubes were placed in racks in the incubator, and so arranged that they could be examined without disturbing them. By this means the statement of Clairmont was confirmed, in which he notes that there is practically always a pellicle of more or less delicacy, easily broken up and not re-formed. This pellicle varied markedly in amount and consistency with different samples of bouillon. In some cases the growth was much more marked than in others, and the amount of pellicle varied directly with the profusion of the growth, as might, indeed, be expected. Where the surface growth was marked, there was usually a white ring at the margin.

In all cases the medium was uniformly clouded, and a flocculent, but rather heavy, sediment was formed in varying amount. On shaking, this was readily diffused through the medium, and did not settle completely for some hours. The consistency of the bouillon was not changed, save that it was made slightly denser by the presence of the sediment, except in No. 9, and sometimes in B. rhinoscleromæ, in which the fluid showed marked viscidity.

INDOL REACTION.

As recommended by Theobald Smith, tests for indol were made in dextrose free bouillon, determined by control to be free from indol. The cultures were kept in the incubator for eight days, and tested by the method recommended by Lehmann and Neumann. To each tube $\frac{1}{2}$ volume of 10 per cent. sulphuric acid

was added, and the mixture heated to 80° C. It was then allowed to stand for a few minutes, in case the reaction should take place without further treatment. In any case, a drop of ½ per cent. sodium nitrite was added, and one or two more if necessary. The reaction was noted, and further observations were made at the end of twenty-four hours.

In all but three cases—Hopkins No. 6, B. pseudo-pneumonicus, and B. rhinoscleromæ—a positive reaction was obtained, marked in Nos. 5–9, 18, 19, Hopkins Nos. 1, 3, 4, B. sputigenus crassus, B. capsulatus septicus, Pfeiffer, B. ozænæ Abel, B. acidi lactici, and Blumer, and slight in the rest.

In B. acidi lactici, Blumer, and No. 9 there was usually, though not always, a positive reaction without the addition of nitrite. These three organisms have been grouped together because of other similarities, and it is interesting that they should conform also in this. The reaction was not absolutely constant in any case, and Clairmont was unable to obtain it at all; so too much stress must not be laid on it.

FERMENTATION TESTS.

In this part of the work dextrose free bouillon was used as a basis. It was prepared according to the method of Theobald Smith, and titrated to + 1.5. To the media thus prepared 1 per cent. of the various carbohydrates tested was added, and the mixtures when placed in fermentation tubes and sterilized by the fractional method. All chemicals were chemically pure. The following were the carbohydrates used:

Monosaccharids	Dextrose and levulose
Disaccharids	Saccharose, lactose, maltose
Pentoses	Arabinose
Triatomic alcohol	Glycerin
Hexatomic alcohol	Mannite

In the first series of tests each sugar was taken up separately, and all organisms inoculated into the same batch of bouillon. Later, each organism was inoculated into all the sugars, made up with one batch of bouillon. This was done because well-marked differences occurred in different batches of bouillon.

The fermentation tubes were inoculated from recent agar cultures, and kept in the incubator at body temperature. They were examined daily as long as gas formation continued, and daily record was made of the amount of gas, the reaction of the open bulb, and the appearance of the media in the stem. When no gas had been formed for twenty-four hours, the equation $H : CO_2$ was estimated with 2 per cent. NaOH in the usual manner. In all cases where gas formation was weak, or where no gas was formed in one or more of the substances tested, or where the organisms had been a long while without passage through animals, inoculations were made in an attempt at standardization. Unfortunately, most of the cultures from Prague had apparently quite lost their pathogenicity, and, in spite of large and repeated doses, would no longer kill animals. All cases which failed to make gas in one or more substances were tested a number of times with different samples of bouillon to secure accuracy of results.

As arabinose is not fermented by yeasts, representatives of the different groups were tested in its solutions, and were all readily able to break it up with the formation of gas.

The appended Table I shows the result of the inoculations. As noted above, some of the Cleveland series were so similar that all are not given.

From the standpoint of gas formation the organisms studied fall into three groups:

I. *Those which ferment all the carbohydrates used.*

II. *Those which ferment all but one or two.*

III. *Those which ferment none at all.*

By far the majority fall into the first group, and in the following summary all not mentioned under Groups II and III belong under Group I, and will not be specified. Under Group II we have the following subdivisions:

1. *No gas in lactose.*—None of these coagulate milk. This includes Friedländer, Fasching, B. crassus sputigenus, B. ozænæ Abel, Wright and Mallory, No. 6, Hopkins Nos. 3–6. In this division the first three and the last gave no gas in glycerin or in glycerin agar.

2. *No gas in saccharose.*—All these coagulate milk. This

TABLE 1.

NAME OF CULTURE	AFTER TWO WEEKS			DEXTROSE			LACTOSE			SACCHAROSE			LEVULOSE			MALTOSE			ARABINOSE			MANNITE			GLYCERIN		
	Milk Alk. after Hours	Milk Acid. No Coag.	Milk Coag. in Hours	Gas in Closed Arm	Reaction of Bulb	Cloud in Arm without Gas	Gas in Closed Arm	Reaction of Bulb	Cloud in Arm without Gas	Gas in Closed Arm	Reaction of Bulb	Cloud in Arm without Gas	Gas in Closed Arm	Reaction of Bulb	Cloud in Arm without Gas	Gas in Closed Arm	Reaction of Bulb	Cloud in Arm without Gas	Gas in Closed Arm	Reaction of Bulb	Cloud in Arm without Gas	Gas in Closed Arm	Reaction of Bulb	Cloud in Arm without Gas	Gas in Closed Arm	Reaction of Bulb	Cloud in Arm without Gas
Fasching, Král	48	..	..	.45	Ac.	..	.00	Alk.	48	.15	Ac.	..	.12	Alk.	..	.43	Alk.	..	.27	Alk.	..	.12	Ac.	..	.00	Alk.	48
B. aërogenes Král	..	..	72	.30	Ac.	..	.62	Alk.	..	.50	Ac.	..	.18	Alk.	..	.07	Ac.	..	....	..	..	.80	Ac.	..	.78	Ac.	..
B. cras. sput. Král	..	++	..	.27	Ac.	..	.25	Ac.	..	.28	Ac.	..	.24	Ac.	..	.50	Ac.	..	....	..	..	.24	Ac.	..	.00	Alk.	48
B. pseudo-pneum. Král	..	..	..	.35	Ac.	..	.00	Alk.	24	.68	Alk.	..	.21	Ac.	..	.30	Alk.	..	....	..	..	.25	Ac.	..	.00	Alk.	24
B. Pfeiffer, Král	..	..	72	.80	Ac.	..	.47	Ac.	..	.74	Alk.	..	.65	Alk.	..	....	..	..	....	..	..	.80	Ac.	..	.87	Ac.	..
B. ozænæ Abel, Král	72	..	..	.18	Alk.	..	.00	Alk.	24	.20	Ac.	..	.12	Alk.	..	.45	Alk.	..	....	..	..	.00	Alk.	48	.00	Alk.	24
B. rhinoscleromæ Král	72	..	48	.00	Alk.	96	.00	Alk.	24	.00	Alk.	96	.00	Alk.	48	.00	Alk.	96	.00	Alk.	48	.00	Alk.	24	.00	Alk.	24
B. acidi lactici Král	..	..	48	.33	Ac.	..	.20	Ac.	..	.00	Alk.	24	.33	Ac.	..	.50	Alk.	..	....	..	..	.40	Ac.	..	.15	Alk.	..
B. pneum. Friedl., Král	..	+	..	.30	Ac.	..	.00	Alk.	48	.40	Ac.	..	.27	Ac.	..	.42	Alk.	..	....	..	..	.35	Ac.	..	.00	Alk.	48
B. m. c. Hopkins, 1	..	..	48	.45	Ac.	..	.30	Ac.	..	.32	Ac.	..	.36	Ac.	..	....	..	..	....	..	..	.52	Ac.	..	.68	Alk.	..
B. m. c. Hopkins, 2	..	..	48	.35	Ac.	..	.45	Alk.	..	.35	Ac.	..	.35	Ac.	..	....	..	..	....	..	..	.42	Ac.	..	.72	Alk.	..
B. m. c. Hopkins, 3	..	+	..	.55	Ac.	..	.00	Alk.	24	.20	Ac.	..	.15	Ac.	..	.12	Alk.	..	....	..	..	.70	Alk.	..	.55	Alk.	..
B. m. c. Hopkins, 4	..	..	48	.50	Ac.	..	.00	Alk.	24	.38	Ac.	..	.52	Alk.	..	.62	Alk.	..	.37	Ac.	..	.32	Ac.	..	.32	Alk.	..
B. m. c. Hopkins, 5	..	++	..	.00	Alk.	96	.00	Alk.	Ab.	.00	Alk.	96	.00	Alk.	48	.00	Alk.	48	.00	Ac.	96	.00	Alk.	Ab.	.00	Alk.	Ab.
B. m. c. Hopkins, 6	..	+	..	.30	Ac.	..	.00	Alk.	24	.40	Alk.	..	.12	Ac.	..	.50	Alk.	..	.35	Alk.	..	.38	Ac.	..	.00	Alk.	24
B. m. c. Blumer	..	..	48	.45	Ac.	..	.25	Ac.	..	.00	Alk.	72	.32	Ac.	..	.70	Alk.	..	.45	Alk.	..	.62	Ac.	..	.30	Alk.	..
B. m. c. Howard	..	..	72	.75	Ac.	..	.44	Alk.	..	.70	Alk.	..	.45	Ac.	..	....	..	..	....	..	..	.56	Alk.	..	.70	Alk.	..
B. m. c. Wright and Mallory	..	+	..	.65	Ac.	..	.00	Ac.	36	.38	Ac.	..	.45	Alk.	..	.18	Ac.	..	.18	Ac.	..	.25	Alk.	..	.20	Alk.	..
B. m. c. Cleveland, 1, 13	..	..	48	.45	Ac.	..	.26	Ac.	..	.20	Alk.	..	.18	Ac.	..	.45	Ac.	..	....	..	..	.35	Ac.	..	.05	Alk.	..
B. m. c. Cleveland, 2	..	..	48	.70	Ac.	..	.40	Ac.	..	.70	Alk.	..	.40	Ac.	..	....	..	..	....	..	..	.65	Ac.	..	.80	Alk.	..
B. m. c. Cleveland, 3, 14–17	..	..	48	.80	Ac.	..	.75	Ac.	..	.45	Alk.	..	.50	Ac.	..	.60	Alk.	..	.75	Ac.	..	.70	Alk.	..	.70	Alk.	..
B. m. c. Cleveland, 4	..	..	48	.80	Ac.	..	.50	Ac.	..	68	Alk.	..	.48	Ac.	..	....	..	..	....	..	..	.76	Alk.	..	.75	Alk.	..
B. m. c. Cleveland, 5, 18, 19	..	..	48	.80	Ac.	..	.51	Ac.	..	.81	Alk.	..	.42	Alk.	..	.60	Alk.	..	.80	Alk.	..	.60	Alk.	..	.88	Alk.	..
B. m. c. Cleveland, 6	..	+	..	.80	Ac.	..	.00	Alk.	48	.38	Ac.	..	.60	Alk.	..	.45	Alk.	..	....	..	..	.50	Alk.	..	.00	Alk.	24
B. m. c. Cleveland, 7	..	..	72	.30	Ac.	..	.43	Ac.	..	.62	Alk.	..	.50	Ac.	..	....	..	..	....	..	..	.65	Alk.	..	.58	Ac.	..
B. m. c. Cleveland, 8	..	..	48	.38	Ac.	..	.25	Ac.	..	.45	Alk.	..	.30	Ac.	..	.70	Ac.	..	....	..	..	.45	Ac.	..	.85	Ac.	..
B. m. c. Cleveland, 9	..	..	24	.22	Ac.	..	.17	Ac.	..	.00	Alk.	72	.18	Ac.	..	.15	Ac.	..	.46	Ac.	..	.29	Ac.	..	.46	Ac.	..
B. m. c. Cleveland, 10	..	..	48	.78	Ac.	..	.45	Ac.	..	.74	Ac.	..	.45	Ac.	..	....	..	..	....	..	..	.80	Ac.	..	.85	Ac.	..
B. m. c. Cleveland, 11	..	..	48	.85	Alk.	..	.80	Alk.	..	.80	Alk.	..	.70	Alk.	..	....	..	..	....	..	..	.85	Alk.	..	1.00	Alk.	..
B. m. c. Cleveland, 12	..	..	48	.65	Ac.	..	.35	Ac.	..	.55	Ac.	..	.60	Ac.	..	....	..	..	....	..	..	.80	Ac.	..	.80	Ac.	..

NOTE.—In the above table the decimals under "Gas in Closed Arm" indicate the proportion of the arm in which bouillon was displaced by gas. Under the head "Reaction," "Ac." = acid, "Alk." = alkaline. For convenience, amphoteric reactions have been classed as alkaline. The figures under "Cloud in Arm without Gas" indicate the number of hours of the persistence of the cloud.

includes Blumer, B. acidi lactici, No. 9. The last gave no gas in glycerin.

Under Group III there are only two organisms, Hopkins No. 5 and B. rhinoscleromæ. B. pseudo-pneumonicus, when received from Král, fermented nothing, but after passage through animals became more active and fell under Group I.

The *relative amount of gas formed* was extremely variable in successive inoculations of the same organism. Some samples of meat seemed more favorable than others for the development, and induced the formation of a larger amount of gas. This continued true, though, as far as could be seen, the method of preparation was identical, and the only inconstant factor was the meat. In some cases the amount of gas formed by the same organism in successive samples of meat bouillon varied as much as between 10 per cent. and 80 per cent. of the closed arm or stem. More than this, successive inoculations into a series of sugars sometimes gave more gas in dextrose, less in lactose, and more in saccharose than in the former trial, or some other similar variation. For this reason statements as to the relative amounts of gas formed can be general only.

Strong, in his Group I (Friedländer, Wright, and Mallory, B. ozænæ Abel, B. sputigenus crassus, and probably rhinoscleromæ), finds the gas in saccharose about one-half the closed arm, in dextrose about one-third, and in lactose about one-third or less. This corresponds fairly well with our averages, except that in this group the gas in lactose is nil.

In his Group II (Pfeiffer, B. aërogenes, Kruse), Strong states that gas is made in all three sugars, in about equal amounts, perhaps least in dextrose. In the series under discussion B. aërogenes agrees fairly well, but Pfeiffer constantly shows twice as much gas in dextrose and saccharose as in lactose. The organisms obtained in Cleveland, which, with two exceptions, belong to this group, sometimes make as much as 80–90 per cent. of gas in dextrose, and only 20 per cent. in lactose and saccharose.

This variation does not interfere in any way with the more absolute classification based on the presence or absence of gas in these solutions.

In all of his cultures Strong obtained fermentation of saccharose, and considered this sugar as of least value, while in the present work three organisms can be sharply separated from the rest by this means.

Leaving aside for the present the most-used carbohydrates, the others may be dismissed quite briefly.

Levulose is of little value as a factor in differential diagnosis. Gas is formed up to about 40 per cent. of the closed arm, by all organisms which ferment dextrose. The equation $H : CO_2$ varies between $\frac{3}{1}$ and $\frac{1}{1}$.

Mannite may be dismissed with about the same statement. The gas usually fills about 50–80 per cent. of the closed arm; $H : CO_2 = \frac{4}{1}$ to $\frac{2}{1}$.

Maltose is also readily fermented, in a manner almost identical with mannite, all dextrose fermenting organisms making 50–80 per cent. of gas, with the equation $H : CO_2 = \frac{2}{1}$ to $\frac{1}{1}$.

Arabinose is similar in its reactions. It was not tried in all cases, but in several representatives of each class, and was found to be fermented by the dextrose fermenters, with formation of from 30 to 80 per cent. of gas in the closed arm, $H : CO_2$ being about $\frac{1}{1}$. The fact that it is not fermented by yeasts made it of interest in this line, but it seems to be of no more value than the three preceding.

Glycerin.—As a general thing, it is easily fermented, but when an organism begins to weaken in its fermentative powers, this seems to be the first to resist. In the present series, Friedländer, Fasching, B. ozænæ Abel and B. sputigenus crassus, which form no gas in lactose, and B. pseudo-pneumonicus, which do ferment lactose, form no gas in glycerin. It is noteworthy in this connection that Fricke mentions that he was unable to obtain gas in glycerin agar with his culture of Friedländer.

No. 6, the only one in the series which did not ferment lactose, had no effect on glycerin, and No. 9, which did not ferment saccharose, was similarly inactive. On the other hand, the Hopkins cultures, while unable to ferment lactose, fermented glycerin readily. This suggests to us that the lack of fermentation of glycerin may be a loss, more or less temporary, rather than a

definite characteristic. Of course, it may be said that the lack of lactose or saccharose fermentation may be of similar origin, but the fact observed in the class in bacteriology, that occasionally gas forming cultures of various groups suddenly refuse to make gas in glycerin, and the necessity of a good deal of further and cumbrous subdivision of the classes made, if this be accepted as a differentiation, strengthen my belief in the irregularity of the glycerin reaction. The amount of gas made varied in wide range, from a mere trace in some of the cultures received from Král, weak also in fermentation of other carbohydrates, to a full 100 per cent. of the closed arm in No. 11. $H : CO_2 = \frac{4}{1}$ to $\frac{3}{2}$.

The reaction of the open bulb, taken at intervals of twenty-four hours, in the inoculated tubes of the different sugars, was somewhat variable, as seen in the table. As noted by Clairmont, Fricke, and others, there is an alkali formation, which is either coincident with the acid formation, or follows rapidly upon it. After fermentation and acid production cease, this continues and may overneutralize the acid already formed. This alkali production varies a good deal, as may also be noted from the table, in which the reactions are taken after gas formation has ceased for twenty-four hours, or after six days in those cases where there was no gas formation at any time.

In the organisms which ferment dextrose and lactose with the formation of acid, there is usually enough acid formed to counteract the alkali in the open bulb, and the reaction remains the same as the original, unless the test is made some time after the cessation of gas formation, in which case the reaction of the bulb is always alkaline. In the other carbohydrates, the acid formation is often inadequate for the neutralization of the alkali, and so it is not infrequent to find alkali in the bulb while the production of gas is still active. In saccharose and in glycerin, even when the gas formation is so active as almost to empty the closed arm of fluid in twenty-four hours, this is often seen. Strong, Clairmont and others have estimated the acid formation, stating that $\frac{1}{30}$ c.c. of $\frac{n}{1}$ NaOH is required to neutralize 1 c.c. of a three-day culture. As a usual thing, the fermentation proceeds about that long, and in such cases my results and theirs agree fairly

well; but in some cases gas production continues for as long as ten days, and the continued formation of alkali in the open bulb leads to its diffusion through the fluid in the closed arm, with a consequent change in the reaction.

Taking up more specifically the organisms which failed to make gas in one or more sugars, it is important to determine whether they affect these sugars at all. Anaërobic growth cannot take place without the presence of some material which can be disassociated by the organism with the formation of oxygen, and in general the carbohydrates are the most suitable for this purpose. It is true that in the fermentation tube the condition is not one of true anaërobiosis, but this is approached to a fair degree, so that obligate aërobes will not develop in the closed arm. Growth in the closed arm is indicated by the presence of a cloud, and also by the formation of chemical products due to the growth of the organisms. The reaction of the closed arm is very difficult to obtain with accuracy, and small quantities of acid are almost certain to be obscured by the alkali formation noted in the bulb. Our observations are therefore confined to the *presence or absence of visible growth in the closed arm.*

In the organisms which did not form gas in *lactose*, No. 6, Hopkins No. 4, and Wright and Mallory in the stock cultures, gave a slight cloud for the first twenty-four hours, which disappeared later; but after these organisms were passed through guinea pigs, the growth was practically strictly aërobic. Hopkins No. 3, No. 6, Fasching, B. sputigenus crassus, and B. ozænæ Abel showed a slight cloud for the first twenty-four hours, and none thereafter. Friedländer from Král gave a cloud which persisted for forty-eight hours, and then faded away. Of the organisms which formed no gas in *saccharose*, all three gave a diffuse cloud in the closed arm in twenty-four hours, which was less in forty-eight hours and practically absent after that. In all these cases the reactions of the open bulb were either alkaline or amphoteric, and there was not enough acid in the closed arm to neutralize the alkali in a mixture of the two. These results indicate a *measure of growth in the closed arm.* The amount in no case was large, and no chemical determinations were made as to

the products of growth. It is possible, as noted before, that there might have been traces of dextrose in the bouillon, either from insufficient treatment with the colon bacillus, or from slight impurities in the sugars used. In the absence of accurate chemical tests, this cannot be absolutely determined. In any case, the constant characteristic of gas or no gas was not altered in any way.

In summation of the work on fermentation, it may be stated that in study of the organisms of this group the only ones of the carbohydrates which are of any value in differentiation are lactose and saccharose. Dextrose is valuable in the general classification, but not as a means of separation of the various members. Glycerin may also be of some value at times.

The only definite and constant division which seems justifiable from the study of the cultural characteristics is made by the separation of those organisms which ferment all carbohydrates from those which fail to ferment one or more. This agrees with Strong's ideas, but to his divisions must be added that one which does not ferment saccharose.

The rhinoscleromæ which came from Prague is apparently hopelessly degenerated, as it consistently refuses to make gas in anything. Strong had the same difficulty with his culture from the same source, but records that in another culture obtained elsewhere, and of more recent origin, the organism failed to make gas in lactose, while making it in dextrose and saccharose, and is probably to be considered as belonging in the same division as Friedländer.

In all the forms which were pathogenic, capsules were present in the animal body, and as a usual thing in milk, though in this medium they were sometimes much harder to demonstrate than in others. Many of the text-books say nothing about the capsules of B. acidi lactici, but these were readily seen in milk. The form of the capsule and its reaction to stains did not seem to offer any satisfactory means of classification.

Passing on from the cultural characteristics to other means by which classification of this has been attempted, there remain pathogenicity, and its natural sequences, immunization and agglutination.

Pathogenicity.—This has been taken up by a number of observers, working with the usual laboratory animals, and quite a number of classifications have resulted. The most extensive, as well as the most recent, articles on this subject are those of Fricke, in 1895–96, and Clairmont in 1901–02, and their conclusions, based on their experiments, will be found in Table II.

TABLE II.

Pathogenicity.

Name of Organism	Clairmont			Fricke—Summary			Perkins		
	M.	G. P.	R.	M.	G. P.	R.	M.	G. P.	R.
Friedländer	+	+	−	+	+/−	−	..	+	..
Pfeiffer	+	+	−	+	+	−/+ in large amount	..	+	..
Fasching	+	−	−	+	−	−	..	+	..
B. capsulatus septicus (P. proteus hominis)	−	−	−	+	+/−	+/−	..	−	−
B. ozænæ Abel	+	−/+	−	+	−	−	..	−	−
B. rhinoscleromæ	−	?	−	+	+/−	−	..	−	−
B. crassus sputigenus (Kreibohm)	..	..	..	..	+	−	..	−	−
B. aërogenes	−	−/+	+/−	..	..	..	..	+	..
Wright and Mallory	..	..	..	+	+	+	..	+	..
Blumer	..	..	..	..	..	..	..	+	..

NOTE.—In the above table "M" = white mouse, "G. P." = guinea pig, and "R" = rabbit. The plus sign indicates the death of the animal, the minus sign its survival, and the combination of the two a variable pathogenicity, the more common result being uppermost. The "?" under B. rhinoscleromæ indicates that in the cases where death occurred cultures were negative. The mouse inoculations are subcutaneous, the guinea pig and rabbit intraperitoneal. In my own series of B. aërogenes the pathogenicity was variable, some killing rapidly, some slowly, and some not at all. The pathogenicity in my series was tested only as a means of standardizing the weaker organisms, and so is less complete than some of the others.

Fricke divides his organism into two groups, one of which is pathogenic for white mice, and little or not at all for guinea pigs, and another which is non-pathogenic for mice and kills guinea pigs readily. Clairmont divides his into three groups according

to their pathogenicity for mice, guinea pigs, and rabbits. The results, when tabulated, show that organisms from the same source may have diametrically opposite qualities, and Fricke gives no comparison of organisms described by others except by quotations from the original articles. Clairmont considers Friedländer, Fasching, B. capsulatus septicus, and Pfeiffer. He finds Fasching pathogenic for white mice only, Pfeiffer and Friedländer pathogenic for mice and rabbits, and B. capsulatus septicus not pathogenic. In my inoculations with the same organisms, obtained from the same source, Král's laboratory, R. capsulatus septicus was found to be non-pathogenic for guinea pigs, while Friedländer, Pfeiffer, and Fasching were readily and rapidly pathogenic. The single fact of the marked difference in the pathogenic properties of Fasching here and abroad, though the culture came from the same source, if accuracy in technic be admitted, is sufficient to throw out any classifications based on pathogenesis alone. Different breeds of the same animal may vary markedly in their reaction to bacteria, and it is also an accepted fact that organisms which are classed under the same head—*e. g.*, different strains of Streptococcus pyogenes, or of B. coli—may differ greatly from one another in their pathogenicity. For these reasons, classification on this basis seems unsatisfactory.

IMMUNITY AND AGGLUTINATION.

A number of attempts have been made to immunize animals against the organisms of this group, and to produce a protective serum. Among the earliest of these, Howard succeeded in immunizing guinea pigs against ordinarily fatal doses. Injection of their serum had, however, no protective effect.

Clairmont goes into the subject extensively, with discussion of previous work. The usual methods of immunization were used, and Pfeiffer's test as well, but though in many cases immunization was successful, the writer was led to the following conclusions:

"Die serodiagnostische Methode war damit als unbrauchbar erwiesen, und es musste auf jene schon oft benutzte Momente zurückgegriffen werden, um durch deren Erweiterung und genaue Feststellung unter möglichst gleichen Bedingungen die misslun-

gene Differenzirung nochmals zu versuchen. Wenn es sich früher als zweckmässiger erwiesen hatte, von bestimmten, aber nicht bindenden, vom Fundort genommenen Bezeichnungen der Stämme auszugehen, so musste jetzt jeder einzelne Stamm als solcher beschrieben werden, da ja die Bakterien in verschiedener Localität doch identisch sein konnten."

It seems as if the present knowledge of technic had been almost exhausted in the effort to separate the various members of the group one from another. The marked disagreement of different authors as to the appearance of the growths on agar, blood serum, gelatin, and potato, and in ordinary bouillon, show that there are no constant characteristics on these media except for the group as a whole. As just noted, pathogenicity and immunization are too variable to admit of making any hard and fast lines. The morphology, inasmuch as one of the main characteristics of the group is its pleomorphism, is of no great aid to us.

These close interrelations suggest the idea, brought out by several observers, that all these organisms may be descendants from one common stem. Some think this to be the Friedländer type, but on theoretical grounds it seems more probable that, if there is an original type, it is rather the "aërogenes" type, which ferments all carbohydrates, as it indicates a loss of power in the succeeding members of the group, rather than the acquisition of additional powers. Then, too, if Friedländer is the original type, the group which ferments lactose, but not saccharose, must have changed in a very roundabout way, or must bear no relation to the general group, to which, however, it is related closely both by all its cultural reactions and by its morphology.

After consideration of all these things, the only characteristic which has been found to be constant, and which is becoming daily of more importance in the classification of species, relates to the fermentation of chemically pure sugars, of which, as noted above, lactose and saccharose are most important as regards this group. Classification on this basis gives us three classes, easily distinguished from one another by the methods at hand in every well-equipped laboratory. Beginning with the largest and most active class, we have the following:

I. *All carbohydrates fermented with the formation of gas.*

II. *All carbohydrates, except lactose, fermented with the formation of gas.*

III. *All carbohydrates, except saccharose, fermented with the formation of gas.*

If this be admitted, it becomes necessary to find suitable names for these three divisions. Inasmuch as the best-known and earliest described of the organisms which fall into the first class is B. lactis aërogenes, its name should obtain. The name must conform to the rules of nomenclature, and accordingly that noted in Migula has been selected — Bacterium aërogenes, Migula, 1900. The prototype of the second class in Friedländer and the name under acceptance for that organism, under the rules above noted, is Bacterium pneumonicum, Migula, 1900. The third class has as its prototype B. acidi lactici, which is a satisfactory name for the organisms of this type.

B. acidi lactici has been classed by some with the colon group, but by most with B. aërogenes, usually with slight description. Hueppe, in the original description, described spores, but these have not been seen in any of the cultures by that name, received from various sources. Capsules, as noted above, were constant. The organism noted by Ford under the head of Bacterium duodenale answers exactly to the description of No. 9, and Blumer, as well as Bact. acidi lactici. Blumer's organism came from a case of septicemia, originating in acute enteritis; No. 9 came from a case of acute appendicitis; and Ford's Bact. duodenale came from the upper part of the intestine as a usual thing. The fermentation characteristics are very constant, inoculations of Blumer made in July, 1903, giving the same reactions as described by him in 1901.

The final classification is, then, as follows:

I. *Bacterium aërogenes.*—Under this head are included Bacterium aërogenes, B. capsulatus septicus, Pfeiffer and Howard, also Hopkins Nos. 1, 2, and all my series except No. 6.

II. *Bacterium pneumonicum.*—Under this head come Friedländer, Fasching, B. sputigenus crassus, B. ozænæ Abel, Wright and Mallory, Hopkins Nos. 3, 4, 6, and No. 6 of my series. B. rhinoscleromæ probably comes within this division.

III. *Bacterium acidi lactici.*—Under this head come B. acidi lactici, Blumer, and No. 9 of my series.

SUMMARY AND CONCLUSIONS.

1. The name *Bacillus mucosus capsulatus* includes a large number of organisms described under various names, closely related morphologically and culturally, but differing in certain characteristics.

2. Animal inoculation and immunization and agglutination show results which are far too variable to admit of using them as means for classification.

3. Growths on the various ordinary laboratory media are subject to such great variations that they are also unavailable for purposes of classification.

4. Experiments in fermentation show constant results which may be used as a basis for the separation of the different members of the group, according to their ability to ferment lactose and saccharose.

5. The organisms of this group are widespread, and have been found in almost all pathological lesions which may be caused by bacteria.

6. Infections due to this group are exceedingly frequent in Cleveland, and appear to be due almost exclusively to the members of the Bact. aërogenes division.

7. These organisms may form gas from the body carbohydrates, either during life or immediately after death.

8. The portal of entry appears to be the nose and mouth, and secondarily the digestive tract.

It will be noted that, besides B. rhinoscleromæ, another organism, Hopkins 5, is described as forming no gas. In this case the organism was said by Dr. Harris, from whom it was obtained, to have formed gas at a previous time. In July, 1903, in spite of attempts to rejuvenate it, it was still unable to ferment the sugars. This, then, seems to be a case of powers lost, in a way similar to that seen in B. rhinoscleromæ; but another organism obtained through the courtesy of Dr. Blumer, and coming from a throat, was unable to grow in the closed arm at any time, being

apparently a strict aërobe, and was entirely non-pathogenic. Another similar organism, obtained from a similar source, came under my observation since the preparation of this paper, and the point has been raised whether organisms such as these, which possess the morphology, staining, and cultural reactions of the group, but have neither pathogenicity nor power to ferment carbohydrates, should be placed in a separate group.

Since, however, we have organisms which have once been able to ferment and now are no longer able to do it, it seems to me more natural to suppose that these non-fermenters belong to one of the regular groups, and have lost their chemical activities as well as their pathogenicity. It is quite possible that further work may show that such organisms should be placed in a section by themselves, but at present I will content myself with the above mention.

In conclusion, I desire to express my thanks to Dr. W. T. Howard, Jr., for the use of the material, and for his many and valuable suggestions in the course of the work. It is also a pleasure to express my acknowledgments to the trustees of the Rockefeller Institute for Research, for the financial assistance given during the course of the work.

REFERENCES.

The literature on this subject has been so thoroughly worked out by Clairmont, and his list of references is so full, that I could have done little more than copy his work. It seemed better, therefore, to note here only such as were actually made use of, and those that Clairmont has omitted:

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NOTE.—This article was prepared for publication July 1, 1902, but has been delayed until now in its appearance. The only article which has appeared since is that of Sachs, *Centralblatt für Bakteriologie*, Vol. XXXIII, Abt. I, p. 657. His work, as he himself admits, adds nothing new to the subject. He found an organism of this type in a case of pyonephrosis, at operation, and made a comparative study. The bacillus coagulated milk slowly, and often made gas in the milk culture. There is no record of the growth or chemical activities in lactose, in glucose, or in saccharose bouillon, but the coagulation and gas formation in milk place it in Class I or in Class III. The absence of records in regard to saccharose makes it impossible to say to which it belongs, but the source and the relative frequency of occurrence of the two make it probable that it should be placed as a member of Class I.